National Wild Fish
Health Survey

California-Nevada Fish Health Center

Annual Report for Fiscal Year 2010

National Wild Fish Health Survey
Annual Progress Report FY 2010

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California-Nevada Fish Health Center

Center staff conducted the National Wild Fish Health Survey (NWFHS) in 2010 by working with partners to collect fish tissue samples and performing laboratory tests for major fish pathogens in accordance with standardized procedures (NWFHS Laboratory Procedures Manual – 2009). This data is entered into a national database and is accessible to the public and resource managers, via the web, and can be viewed at:

http://www.fws.gov/wildfishsurvey/database/

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Overview

The National Wild Fish Health Survey is a program conducted by the U.S. Fish and Wildlife Service's Fish Health Centers to assess the assess the prevalence and distribution of major fish pathogens in wild fish populations. To date, the Center has partnered with numerous federal and state agencies, tribal governments, universities, non-profit and educational organizations and private landowners to collect fish at over 200 collection sites. A total of 17,947 samples have been collected and tested for major fish pathogens over the past 12 years. The sampling effort to date comprises a rich diversity of fish species in California and Nevada and has provided fish health information that did not exist prior to the National Wild Fish Health Survey's inception in 1997.

Each year, the California-Nevada Fish Health Center (Ca-Nv FHC) focuses on specific disease issues that are important in our region such as emerging diseases, health issues in species of special concern, or are important to our partners for management of the fishery resource. Other projects supported by the Survey are reoccurring from year to year in order to provide annual trends in disease prevalence for naturally reproducing broodstock populations, or fish health monitoring of natural juvenile fish, as in the Klamath River basin.

In 2010, the Survey focused on health screenings of imperiled stocks of fish with surveys conducted for the Delta Smelt (*Hypomesmus transpacificus*), Klamath River and the Central Valley Fall-Run Chinook (*O. tshawytscha*). In addition, screenings were performed on the Sacramento River Late fall and Winter-Run (*O. tshawytscha*) and Lost River Sucker (*Deltistes luxatus*). Pathogens screened include Viral Hemorrhagic Septicemia Virus (VHSV), Infections Hematopoietic Necrosis Virus (IHNV), and Infectious Pancreatic Necrosis Virus (IPNV).

As in previous years, we surveyed natural juvenile chinook in the lower Klamath River for the myxosporean parasites *Ceratomyxa shasta* and *Parvicapsula minibicornis*. The ongoing fish health monitoring program provides annual incidence of infection (IOI) data of the myxosporean parasites, by both Quantitative PCR (QPCR) and histology, and support applied research studies and management objectives to recovery chinook and coho in this basin.

Our survey work would be not possible without the support of numerous partners including: California Department of Fish and Game, U.S. Bureau of Reclamation, UC Davis and Oregon State University, USGS, several Fish and Wildlife Offices (Arcata, Yreka, Reno, and Stockton). The Karuk, Hoopa, and Yurok tribes of Northern California and Oregon Department of Fish and Wildlife (ODFW).

Accomplishment Report for 2009 – Pathogen Surveys

Klamath River Fish Health Monitoring Program

Concerns in the Klamath River basin regarding flow allocations and the relationship to disease incidence were heightened during the 2002 adult Chinook salmon (*O. tschawytcha*) fish kill. Many federal, state, local and tribal biologists are conducting research to better understand what biological factors influence the incidence of disease in this river system. Two parasites, *Ceratomyxa shasta* and *Parvicapsula minibicornis*, which occur as dual infections in a large proportion of juvenile salmon, are of special concern. Fish health monitoring studies address the potential disease impacts on survival of natural and hatchery produced out-migrating juvenile Chinook, and provides annual trend data.

Examining gills for Columnaris disease on juvenile Chinook salmon.

Ceratomyxosis has been identified as the most significant disease for juvenile salmon in the Klamath Basin (Foott et al. 1999, Foott et al. 2004). The prevalence of infection (POI) for *C. shasta* and *P. minibicornis* for mixed origin Chinook (hatchery and natural fish) during the 2010 monitoring period (April to August) was 17% and 66% respectively by QPCR. Monitoring of natural fish, sampled and tested prior to hatchery releases indicated that *C. shasta* was first detected by QPCR during early May in the Klamath Estuary to Trinity River, Trinity River to Salmon River, and the Salmon River to Scott River reaches at a POI of 5%, 5%, and 10% respectively. *Parvicapsula minibicornis* was first detected in natural origin Chinook in early May, at POI of 35%, in the upper reach (Salmon River to Scott River), and reached 100% in the Shasta River to Scott River reach in late May. In the lower reaches, *P. minibicornis* POI also reached 100% by June. As in previous years, *P. minibicornis* prevalence rose rapidly, and the majority of natural fish infected with *C. shasta* were also infected with *P. minibicornis*. In the FY2010 sampling season, the incidence of infection indicated that infectivity was very low in comparison to previous years in which monitoring studies were conducted (True et al. 2011).

Surveys of Spawning Adult Salmonids

The completion of Shasta dam in 1945 had an inevitable impact on Chinook salmon and steelhead access to historic spawning habitat. The significant loss of natural spawning areas above the dam was mitigated through the completion of Coleman and Livingston Stone National Fish Hatcheries. Returning Fall Chinook Salmon, Steelhead, Late Fall, and Winter Chinook adults are monitored each year to determine the disease status of adult salmonid populations in the upper Sacramento basin. This report focuses on Winter-run populations.

Winter run Chinook salmon were listed as endangered by California Fish and Game in 1989 and the National Marine Fisheries Service in 1994. Attempts to imprint juveniles reared at CNFH to the upper main-stem Sacramento River were unsuccessful, and in 1997, the Bureau of Reclamation developed a main-stem rearing facility, Livingston Stone NFH, at the base of

Shasta Dam. This facility was successful in producing captive and natural production goals, and ensuring winter run adults returned to the upper Sacramento River. The hatchery's ultimate goal is to recover Winter-run Chinook populations to self-sustaining population levels.

Natural origin Late-Fall adults are captured at the base of Keswick Dam and transferred to LSNFH for egg collection. In 2010, 41 samples were collected from spawned wild fish. Infectious Hematopoietic Necrosis virus (IHNV) was detected in 7 of 8 (88%) pooled kidney samples and 7 of 7 (100%) pooled ovarian samples. *Renibacterium salmoninarum* was not detected in kidney or ovarian fluid tested by QPCR.

Lower Sacramento River Kodiak Surveys

The Ca-Nv Fish Health Center partnered with California Fish and Game to obtain samples of Delta Smelt populations from several sites in the Lower Sacramento River. These efforts were to determine the presence of infectious pathogens (virus, bacteria, or parasite) and tissue abnormalities. An emerging pathogen of concern is Viral Hemorrhagic Septicemia Virus (VHSV). VHSV is a serious systemic disease of fish. The VHS virus is carried by at least 50 species of marine and freshwater fish. The infection is subclinical in some species, but it is associated with severe disease and high mortality rates in others. Clinical infections are economically important in farmed fish, particularly rainbow trout, turbot and Japanese flounder. Outbreaks have also been reported in some wild populations, including Pacific herring and pilchard along the Pacific coast of North America. A total of 79 fish were screened for bacterial and viral pathogens. Sub-clinical *Mycobacterium sp.* infection was detected by PCR. No NWFS reportable viral or bacterial pathogens were detected. Another 72 fish were examined by histological methods for parasite (10% asymptomatic helminth infections) and tissue abnormalities. These fish were apparently healthy as demonstrated by a lack of clinical signs or morbidity (Foott & Bigelow, 2010).

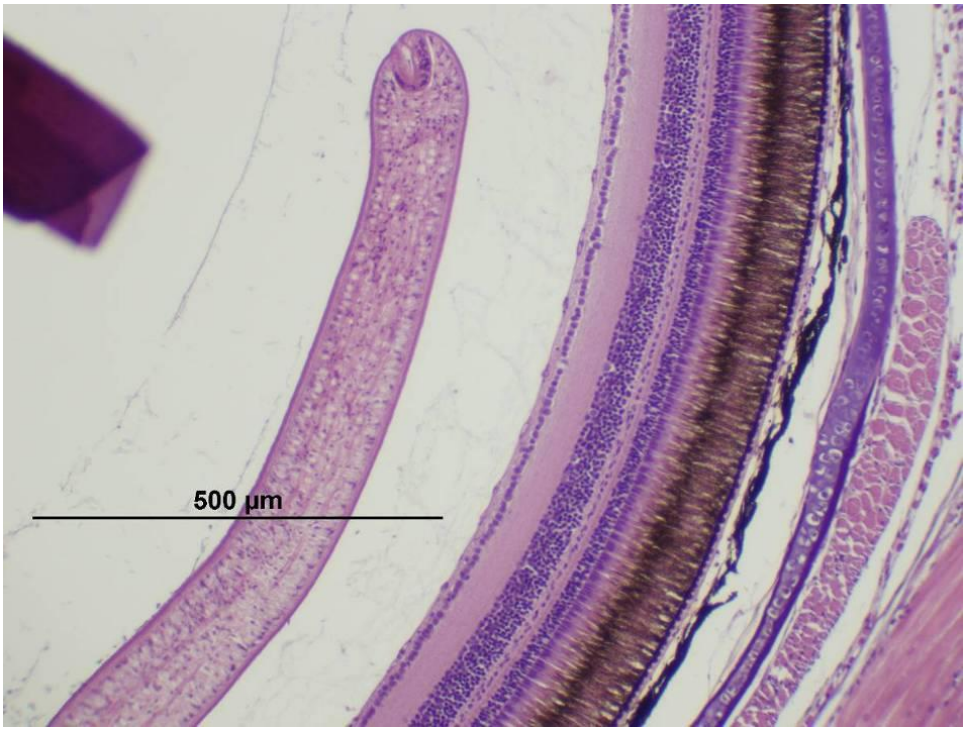
Upper Klamath Lake (Link River dam) and Tule Lake

The FHC performed a health evaluation of juvenile fathead minnows, chub, and suckers (including endangered Lost River and Shortnose suckers) captured by the Bureau of Reclamation in the Link River and from their autumn salvage program. Excluding capture injury at the Link R. rotary screw trap, juvenile suckers were deemed fit enough to survive salvage operations (Foott et al. 2010). The Lost River Suckers, Fathead Minnows, and Blue Chub demonstrated a high incidence of parasite infection (*Trichodina sp.*, *Myxobolus sp.* and various helmiths). Histological evaluation of 25

suckers collected in the autumn salvage demonstrated a low level of parasite infection and no signs of disease (*Trichodina sp., Myxozoan sp.* and trematodes).

Additionally, samples were collected from adult Lost River and Shortnose Suckers at the request of Klamath Falls Bureau of Reclamation and USFWS office to facilitate salvage operations from Tule Lake. Overall fish appeared to be healthy and no viral pathogens were detected.

Figure 11. Trematode (presumptive *Diplostomulum sp.)* within vitreous chamber of chub eye.



Stanislaus River

Fish were surveyed from two out-migrant monitoring sites (RM's 6 and 40) along the Stanislaus River to screen for fish pathogens and disease. Liver, kidney and gill sections were examined for abnormalities indicative of environmental toxicity. No insult or tissue abnormalities were detected in the 109 fish sampled. Only a few (10/74) were found to have bacterial or parasitic infection. While not a threat to fish health at the early stages observed during this study, infections with the kidney parasite *Tetracapsuloides bryosalmonae*, the causative agent of Proliferative Kidney Disease, will progress and could be a significant cause of mortality during migration and transition to sea water (Nichols, 2010).

Laboratory Methods

The methods used in the NWFHS to collect, process, and test fish tissues are standardized throughout the country. The detailed procedures and laboratory protocols can be found in The National Wild Fish Health Survey Procedures Manual (Heil, 2009) at the following websites:

NWFHS http://fisheries.fws.gov/FHC/FHCNational.htm

Bacteriology

A sample of kidney tissue from each fish was streaked onto 100 mm petri plates, or 20 x 125 mm test tube slants, of Brain Heart Infusion Agar (BHIA) and incubated at room temperature for 72 hours. If growth appeared on the BHIA media, isolated colonies were subcultured onto fresh BHIA plates to supply pure cultures of bacteria for phenotypic characterization and presumptive identification. Subcultured isolates were screened for bacterial fish pathogens by standard microscopic characteristics such as Gram stain, morphology, motility and cytochrome oxidase, and appropriate biochemical tests. Bacterial isolates that are ubiquitous in freshwater and without associated clinical signs were identified to a general group, while those that are potential fish pathogens such as *Aeromonas salmonicida*, *Yersinia ruckeri*, or *Edwardsiella tarda* were examined to a presumptive identity. Corroborative testing for positive results included Fluorescent Antibody Testing (FAT), which uses specific antibodies to immunologically confirm the identity of bacterial pathogens.

Renibacterium salmoninarum by ELISA

Kidney tissue from each fish was removed and diluted 1:8 with Phosphate Buffer Saline (PBS) with Tween 20, homogenized, and separated by centrifugation. The samples were then loaded onto 96-well plates and assayed by Enzyme Linked Immunosorbent Assay (ELISA) for the presence of *Renibacterium salmoninarum* antigen. The ELISA tested samples in replicate when the quantity of kidney tissue from individual fish was sufficient. The absorbency values (optical density, OD) were averaged and the distribution of ELISA values for separate groups were evaluated. Individual fish with ELISA OD values greater than 2 standard deviations above the negative reference control OD, and up to 0.499, were defined as low level antigen, 0.500-.999 moderate level, and values of 1.00 or higher were considered high antigen levels. Corroborative testing of ELISA antigen positive test results is required to confirm the presence of *Renibacterium salmoninarum* DNA, and is performed with standard or quantitative Polymerase Chain Reaction (PCR).

Virology

Samples of kidney and spleen, or visceral tissue in the case of smaller fish, were removed from each fish and assayed for the prevalence of Infectious Hematopoietic Necrosis virus (IHNV), Viral Hemorrhagic Septicemia virus (VHSV), Infectious Pancreatic Necrosis virus (IPNV), and Viral Nervous Necrosis (VNNV) using accepted cell culture techniques. Kidney and spleen tissues were tested individually, or from 3-5 fish pooled into one sample. The World Organization for Animal Health accepts the use of SSN-1 cell lines for the detection of VNNV (http://www.oie.int.eng/normes.fmanual/A 00024.htm)

For cell culture assay, tissue samples were weighed and diluted to 1:10 in Hank's Balanced Salt Solution (HBSS) and homogenized with a Stomacher 80 Lab Blender®. Samples were centrifuged at 5000 x g for 15 m and then 1.0 mL of the supernatant was combined with 1mL of HBSS supplemented with antibiotics and antimycotic (200 IU mL-1 penicillin G, 200 IU mL-1 streptomycin, 0.5 µg mL-1 amphotericin B and 40 µg mL-1 gentamycin). Final sample dilutions

of 1:20 and 1:100 were inoculated onto confluent Chinook Salmon Embryo 214 (CHSE-214), Epithelioma Papillosum Cyprinid (EPC), Striped Snakehead (SSN-1), or Bluegill Fry (BF-2) cell lines in replicate onto 48-well plates. The SSN-1 cell line was selected to screen for nodavirus in Delta fish samples and requires L-15 media. Samples were incubated on a platform rocker for 30-60 minutes at 15°C. Wells were supplemented with 0.5ml of liquid overlay which contained Minimum Essential Media with 10% Fetal Bovine Serum (MEM10) or MEM10 with methylcellulose (EPC cell line), and incubated at 15°C for 21 d. Plates were examined bi-weekly for evidence of viral cytopathic effects (CPE), and re-inoculated onto fresh cells if generalized toxicity or suspect CPE was noted. Corroborative testing, if positive, was done by Immunohistochemistry (IHC).

Myxobolus cerebralis (Whirling Disease)

Screening for *Myxobolus cerebralis*, the causative agent of Whirling Disease, was done by Pepsin-Trypsin Digest (PTD) of cranial elements consisting of bone and cartilage. Sampled salmonids were decapitated and the heads grouped into pools of 5 fish, and then frozen until laboratory analysis could be performed. The heads were halved, to provide an archive set for PCR confirmation testing and for PTD testing. Cranial elements were heated in a 60°C water bath for 60 minutes to remove soft flesh. The cranial elements were then ground in a blender and placed in a pepsin solution of 20 mL g-1 of tissue, and incubated at 37°C for 40-60 minutes, depending on sample size. The samples were centrifuged, supernatant removed, and the pellet digested in a solution of trypsin at 20 mL g-1 of tissue. Samples were incubated at room temperature on a rocker plate for 30 minutes. The larger remaining particles were filtered through cheesecloth or large-pore filters, and the samples were centrifuged a final time to concentrate spores, if present. A small amount of water was added to the pelleted preparation to provide adequate solution volume in which the samples could be examined by phase contrast microscopy at 200-400x. Corroborative testing for TPD positive results was done by PCR.

Quantative Polymerase Chain Reaction (QPCR) for *C. shasta* and *P. minibicornis*

Combined intestine and kidney tissues were digested in 1ml NucPrep Digest Buffer containing 1.25 mg/ml proteinase K (Applied Biosystems, Foster City, CA) at 55ºC for 2 hours with constant shaking. A subsample of digested tissue homogenate was diluted 1:33 in molecular grade water and extracted in a 96 well vacuum filter plate system. Extracted DNA was stored at -20ºC until the QPCR assays were performed.

Samples were assayed in Real Time PCR Sequence Detection Systems (SDS) using probes and primers specific to each parasite. The combined tissues were tested for *C. shasta* 18S rDNA using TaqMan Fam-Tamra probe and primers (Hallett and Bartholomew 2006) on the 7300 Sequence Detection System (Applied Biosystems, Foster City, CA). Separately, the combined tissues were tested for *P. minibicornis* 18S rDNA utilizing TaqMan Minor-Grove-Binding (MGB) probe and primers (True et al. 2009) on the StepOne Plus Sequence Detection System (Applied Biosystems Foster City, CA). Reaction volumes of 30µL, containing 5µL DNA template, were used for both assays under the following amplification conditions: 50ºC for 2 min.; 95ºC for 10 min; 40 cycles of 95ºC for 15s and 60ºC for 1 min. Plasmid standards, extraction control and no template control (NTC) wells were included on each assay plate.

References and Additional Reading

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Appendix I - NWFHS SUMMARY TABLE FOR FY 2010

Case #	Date Collected	Location	Species	Partners	Number of Sites	Total Fish	Significant Findings
10-009	9-30-2009	Multiple Sites-NY, NJ, Staten Island	Amphibian-Frogs	Jeremy Feinberg-NYU	13	108	*Batrachochytrium dendrobatidis* 21/108 (19%)
10-026	01-05-10 through 02-17-10 (5 sample dates)	Livingston Stone NFH	Late-Fall Chinook	LSNFH	1	41	IHNV 13/15 samples (87%)
10-008	10-12-2009	Fallen Leaf Lake, CA	Lake Trout	Reno FWO	1	65	
10-007	10-02-2009	Mattole River, CA	Pacific Lamprey	Arcata FWO	1	28	Metacercaria 2/26 (7%)
	10-05-2009	Trinity River	Pacific Lamprey	Arcata FWO	1	28	Metacercaria 3/14 (21%)
10-022	12-1-2009	Upper Klamath Lake, Or	Shortnose/Lost River Sucker	United States Bureau of Reclamation	1	25	See Appendix II for results
10-026	1-13-2010 (5 sample dates)	Sacramento River	Delta Smelt	California Dept. Fish and Game	8	79	See Appendix II for results
10-047	4-14-2010 (3 sample dates)	Stanislaus River, CA	Chinook Salmon	Stockton FWO, FISHBIO, Cramer Fish Sciences	2	109	*F. columnare* 1/62 (2%) *T. bryosalmonae* (PKX) 4/56 (7%) Trematode 1/62 (2%) Amoebas on gill 2/62 (3%) *Ich. multifilis* 1/62 (2%) *Trichodina* 1/62 (2%)

Case #	Date Collected	Location	Species	Partners	Number of Sites	Total Fish	Significant Findings
10-061	4-07-2010	Tule Lake, CA	Shortnose/Lost River Suckers	California Dept. Fish and Game	1	17	Trematode 1/17 (6%) *Lernaea* 4/17 (24%) Nematode 4/17 (24%)
10-080	Multiple sample dates	Trinity River, CA	Chinook Salmon	Hoopa Tribal Fisheries	2	68	*Parvicapsula minibicornis* 3/48 (6%) *Ceratomyxa shasta* 1/48 (2%)
10-081	Multiple sample dates	Klamath River, CA	Chinook Salmon	Arcata FWO, Hoopa/Yurok/ Karuk Tribal Fisheries	5	260	*Parvicapsula minibicornis* 156/260 (60%) *Ceratomyxa shasta* 35/260 (14%)

Appendix 2 – Pathology and Sample Summary Reports to Partners

Case# 10-007 (Mattole River/Trinity River, CA) Pacific Lamprey ammocoetes- *Entosphenus tridentatus*

A total of 28 Lamprey ammocoetes were examined for parasites by histology from the Mattole River. Twenty-eight ammocoetes were also collected from the Trinity River. No significant abnormalities or infections observed in tissues from either site.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
HISTOLOGY:	**Mattole River**				
	Kidney	26 (1p)	2/26[1]	7%	28
Microscopic examination of	Gill	28 (1p)	0/28	0%	28
organs fixed in Davidson's and	Liver	26 (1p)	0/26	0%	28
stained with Hematoxylin and eosin.	**Trinity River**				
	Kidney	14 (1p)	3/14[1]	21%	28
	Gill	28 (1p)	0/28	0%	28
	Liver	22 (1p)	0/22	0%	28

1=encysted metacercaria

Case #10-008 (Fallen Leaf Lake, CA) Adult Lake Trout-*Salvelinus namaycush*

No parasites were observed in any tissues assayed by histology. No reportable bacteria were seen on culturable media.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
HISTOLOGY: Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	Kidney Gill Liver	10 (1p) 4 (1p) 10 (1p)	0/10 0/4 0/10	0% 0% 0%	10 10 10
VIROLOGY: Specific cell lines used: EPC, CHSE-214	Kidney	13(5p)	0/13	0%	65
PARASITOLOGY: Pepsin/Trypsin Digest. Used to test for presence of Myxobolus cerebralis	Head	10 (3p)	0/10	0%	30
BACTERIOLOGY:					
Renibacterium salmoninarum (ELISA)	Kidney	65(1p)	0/65	0%	65
Culturable bacteria on BHIA pure plates	Kidney	30(1p)	0/30	0%	30

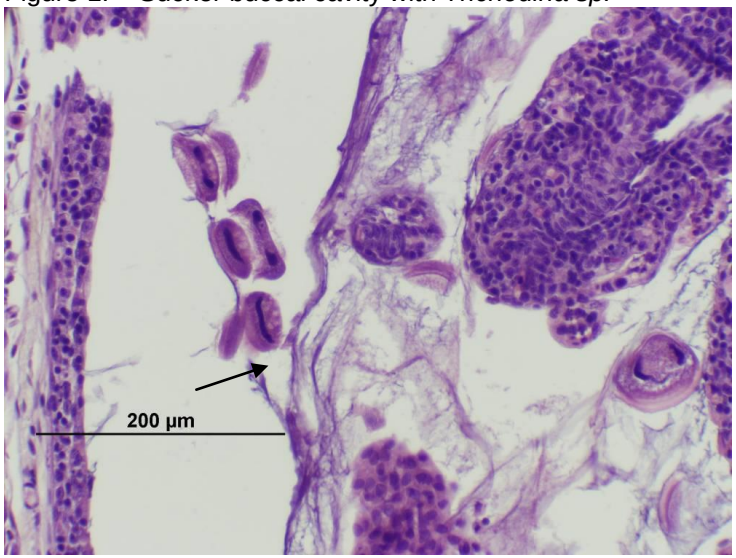
Case# 10-022 (Upper Klamath Lake, J-Canal OR) Juvenile Shortnose/Lost River Suckers- *Chasmistes brevirostris/Deltistes luxatus*

A total of 25 juvenile suckers were examined for parasites by histology. Fish were not identified to species, but were confirmed to be either Shortnose Suckers or Lost River Suckers.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
HISTOLOGY: Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	Kidney	25 (1p)	3,2/25[1,2]	12%,8%	25
	Gill	28 (1p)	1,2,1/28[3,4,5]	4%, 8%, 4%	25
	Intestine	25 (1p)	1,1/25[3,6]	4%, 4%	25
	Adipose	25 (1p)	0/25	0%	25
	Eye	13 (1p)	1/13[3]	8%	25

1=*Trichodina*, 2=Epitheliocystis, 3=Trematode, 4=Myxozoan trophozoite, 5=Hydropic degeneration, 6=Myxozoan cyst

Figure 1. Sucker buccal cavity with *Trichodina sp.*



Case #'s 10-026, 10-032, 10-035, 10-038, 10-042 (Keswick, CA) Adult Late-Fall Chinook- ***Oncorhynchus tshawytscha***

This table summarized results from 5 separate sample dates. 13/15 samples were positive for IHNV. No reportable bacteria were isolated on bacterial media.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
VIROLOGY: Specific cell lines used: EPC and CHSE-214	Kidney/Ovarian Fluid	15(3p)	13/15	87%	41
ImmunoHisto Chemistry (IHC) confirmation	Kidney/Ovarian Fluid	13 (3p)	13/13	100%	39
BACTERIOLOGY: Non-Culturable Bacteria					
Renibacterium salmoninarum (FAT)	Kidney	19(1p)	0/19	0%	19
Culturable bacteria on BHIA pure plates	Kidney	39(1p)	0/39	0%	39

Case #'s 10-029, 10-041, 10-049, 10-050, 10-060, 10-068 (Sacramento River, CA) Delta smelt- *Hypomesmus transpacificus*

This table summarized results from 6 separate sample dates. No viral isolates were observed in the EPC, CHSE-214, Bluegill BF-2, or Snakehead SSN-1 cell lines. No culturable bacteria were seen on BHIA or Middlebrook (7H10 [MB]) plates. Few tissue abnormalities and no overt infectious disease was observed in 72 specimens assayed by histology.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
VIROLOGY: Specific cell lines used: EPC, CHSE-214, BF-2, SSN-1	Kidney	18 (5p)	0/18	0%	79
BACTERIOLOGY: Culturable bacteria on – BHIA pure plates	Kidney	75 (1p)	0/75	0%	79
-Middlebrook pure plates	Kidney	19(1p)	0/19	0%	79
HISTOLOGY: Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	Gill	65 (1p)	0/65	0%	72
	Heart	21 (1p)	0/21	0%	72
	Intestine	72 (1p)	2/73[1]	3%	72
	Stomach	64 (1p)	0/64	0%	72
	Acinar cells	51 (1p)	1/51[2]	2%	72
	Visc. Adipose	62 (1p)	2,1/62[1,3]	3%, 2%	72
	Liver	59 (1p)	1,1,4/59[3,4,5]	2%, 2%, 7%	72
	Spleen	7 (1p)	7/7[6]	100%	72

1=nematodes, 2=granulomatous foci, 3=trematode, 4=liquefactive necrosis foci, 5=inflammatory cell foci, 6=endogenous brown pigment foci

Case#'s 10-047, 10-048, 10-056, 10-057, 10-073, 10-074 (Stanislaus River, CA)
Juvenile Fall Chinook Salmon-*Oncorhynchus tshawytscha*

This table summarized results from 3 separate sample dates. No viral isolates were observed in the EPC or CHSE-214 cell lines. No culturable bacteria were seen on BHIA plates. Few tissue abnormalities and no overt infectious disease was observed in 74 specimens assayed by histology.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
VIROLOGY: Specific cell lines used: EPC, CHSE-214,	Kidney	22 (5p)	0/22	0%	109
BACTERIOLOGY: Culturable bacteria on – BHIA pure plates	Kidney	109 (1p)	0/109	0%	109
Non-Culturable bacteria assayed by FAT	Kidney	17 (1p)	0/17	0%	17
HISTOLOGY: Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	Gill	62 (1p)	1,4/62[1,2]	2%, 6%	74
	Liver	78 (1p)	0/78	0%	74
	Intestine	62 (1p)	1/62[3]	2%	74
	Kidney	56 (1p)	4/56[4]	7%	74
	Acinar cells	55 (1p)	0/55	0%	74
	Adipose	53 (1p)	0/53	0%	74

1=*Flavobacterium columnare*, 2=External parasites including *Ichthyopthirius multifilis*, amoebas, and *Trichodina*, 3=trematode, 4=*Tetracapsuloides bryosalmonae* (causative agent of Proliferative Kidney Disease)

Case# 10-061 (Tule Lake, CA) Adult Shortnose/Lost River Suckers- *Chasmistes brevirostris/ Deltistes luxatus*

No viral isolates were observed in the EPC, CHSE-214, or BF-2 cell lines. No reportable bacteria were seen on culturable media. Fish examined macroscopically exhibited 24% incidence of nematodes in viscera and *Lernaea* on skin.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
VIROLOGY: Specific cell lines used: EPC, BF-2, CHSE-214	Kidney	4(5p)	0/4	0%	17
BACTERIOLOGY: Culturable bacteria on BHIA pure plates	Kidney	17 (1p)	0/1	0%	17
HISTOLOGY: Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin	Liver	15 (1p)	0/15	0%	17
	Gill	15 (1p)	0/15	0%	17
	Eye	2 (1p)	1/2[1]	50%	17
	Kidney	16 (1p)	0/16	0%	17
	Intestine	13 (1p)	0/13	0%	17
GROSS MACROSPOPIC EXAMINATION:	Gill	17 (1p)	0/17	0%	17
	Skin/Fin	17 (1p)	4/17[2]	24%	17
	Viscera	17 (1p)	4/17[3]	24%	17

1=Trematode, 2=*Lernaea*, 3=Nematode

Case #'s 10-080 (Trinity River, Ca) Juvenile Fall Chinook Salmon-*Oncorhynchus tshawytscha*

This table summarized results from multiple sample dates. *Ceratomyxa shasta* was detected in 15% (3/20) of the samples assayed by histology and 2% (1/48) by QPCR. *Parvicapsula minibicornis* was detected in 11% (2/19) of the samples assayed by histology and was found in 6% (3/48) of samples assayed by QPCR.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
HISTOLOGY: Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	Kidney	19(1p)	2/19	11%	19
	Intestine	20(1p)	3/20	15%	20
PARASITOLOGY: CS-QPCR: Detects CS 18s DNA, presumably viable *Ceratomyxa shasta* trophozoites in intestinal tissue	Intestine	48(1p)	1/48	2%	48
PM-QPCR: Detects PM 18s DNA, presumably viable *Parvicapsula minibicornis* trophozoites in kidney	Kidney	48 (1p)	3/48	6%	48

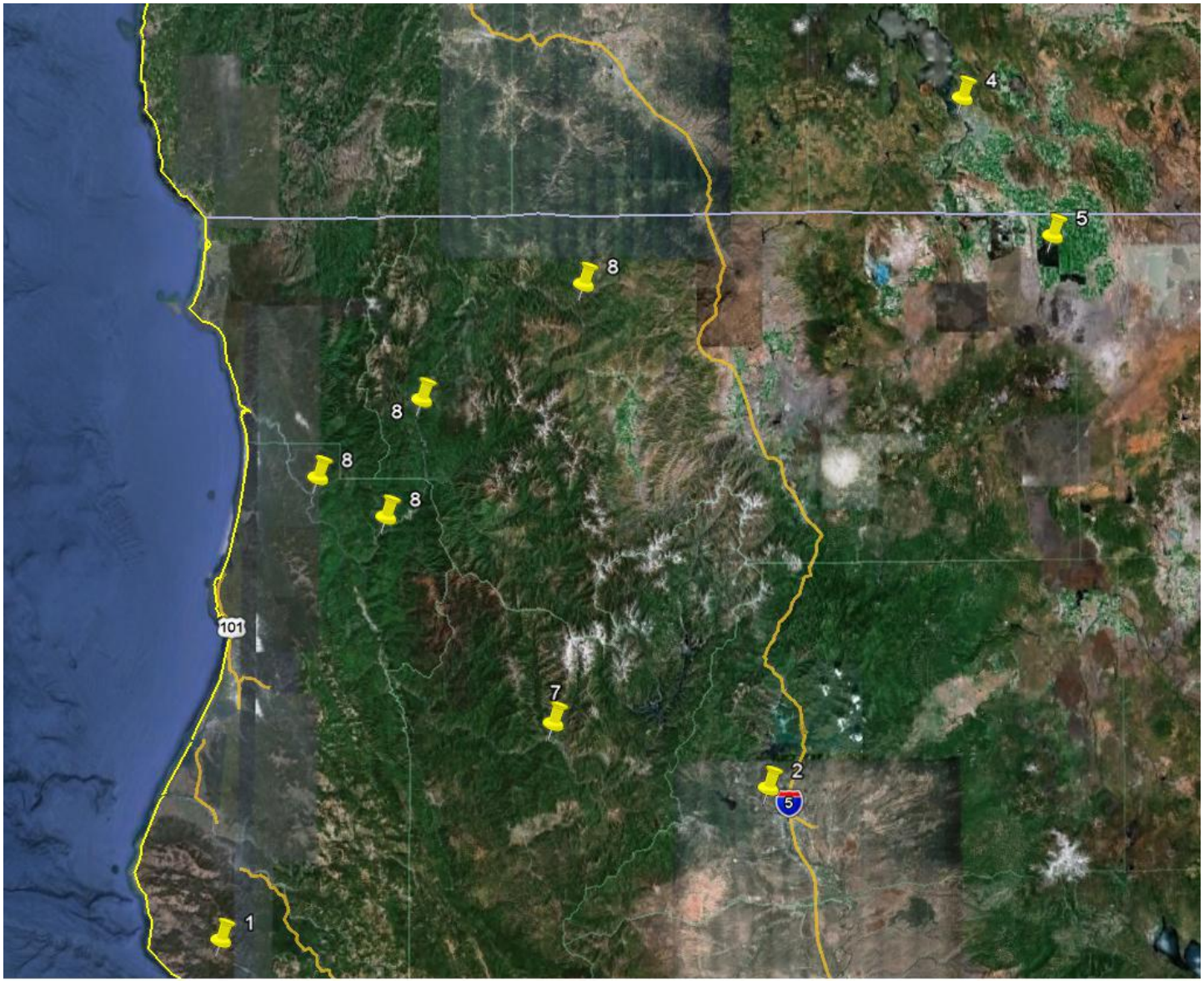
Case #'s 10-081 (Klamath River, CA) Juvenile Fall Chinook Salmon-*Oncorhynchus tshawytscha*

This table summarized results from multiple sample dates. *Ceratomyxa shasta* was detected in 14 % (18/130) samples assayed by Histology and 14% (35/260) by QPCR. *Parvicapsula minibicornis* was detected in 42% (54/129) samples assayed by Histology and 14% (35/260) by QPCR.

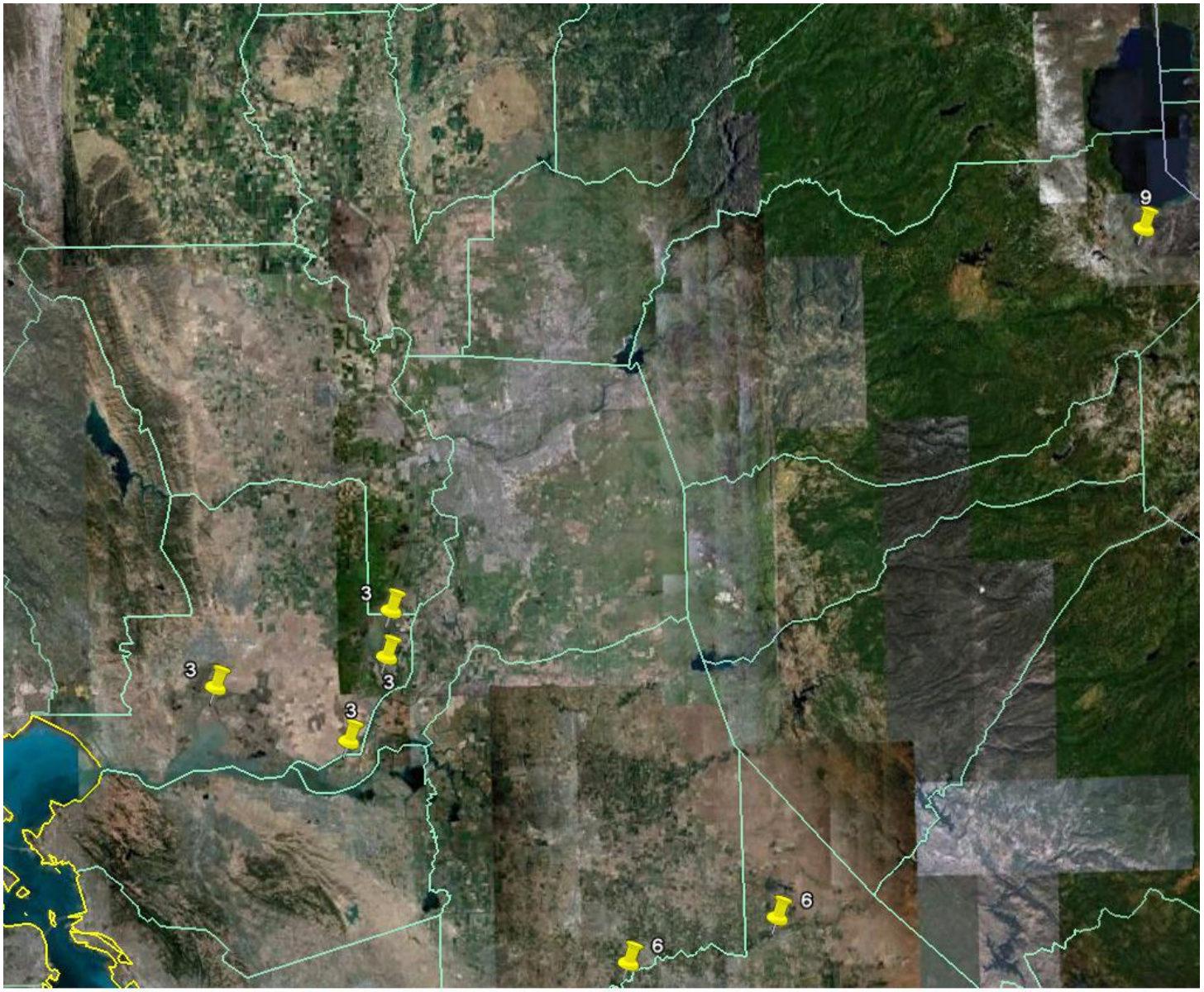
	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
HISTOLOGY: Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	Kidney	129(1p)	54/129	42%	129
	Intestine	130(1p)	18/130	14%	130
PARASITOLOGY: CS-QPCR: Detects CS 18s DNA, presumably viable *Ceratomyxa shasta* trophozoites in intestinal tissue	Intestine	260(1p)	35/260	14%	260
PM-QPCR: Detects PM 18s DNA, presumably viable *Parvicapsula minibicornis* trophozoites in kidney	Kidney	156(1p)	156/260	60%	260

Appendix 3 – Partnerships and Sample Sites

Sample Location		Partner
1.	Mattole River, CA	Arcata FWO-USFWS
2.	Shasta Reservoir, CA	Livingston Stone NFH-USFWS
3.	Sacramento River, CA	California Department of Fish and Game
4.	Upper Klamath Lake, OR	United States Bureau of Reclamation
5.	Tule Lake, CA	California Department of Fish and Game
6.	Stanislaus River, CA	Cramer Fish Sciences
7.	Trinity River, CA	Hoopa Tribal Fisheries, Arcata FWO-USFWS
8.	Klamath River, CA	Arcata FWO-USFWS, Karuk & Yurok Tribal Fisheries
9.	Fallen Leaf Lake, NV	Reno FWO-USFWS



Pins correspond to Appendix 3-Partnerships and Sample Sites



Pins correspond to Appendix 3-Partnerships and Sample Sites